KERATIN BIOSYNTHESIS

II.* EXTRACTION AND CHARACTERIZATION OF NUCLEIC ACIDS FROM WOOL ROOTS

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Summary

Ribosomal RNA (*r*RNA), soluble RNA (*s*RNA), and DNA have been extracted from wool roots in good yield and purity. *r*RNA and *s*RNA were prepared by extracting wool roots with a hydrophilic salt and a phenol–cresol mixture and *r*RNA was selectively precipitated with *m*-cresol. DNA was extracted with a salt having lipophilic and chelating properties, together with the phenol–cresol mixture. Two-stage extraction procedures were essential for the preparation of stable, undegraded nucleic acids.

Rapidly labelled RNA was extracted together with *r*RNA from whole wool roots, polysomes, and from the cell debris remaining after isolating cytoplasmic ribosomes and polysomes. The *r*RNA in the ribosomes and polysomes accounted for 68% of the total, and that from the cell debris 32%. The RNA labelled at short pulse times appeared to be *r*RNA precursor.

I. INTRODUCTION

A study of the action of salts and phenol on mammalian tissues has shown that the ability to separate DNA from protein (Kirby 1957), and RNA from DNA and protein (Kirby 1965), depends on the nature of the salt used. To some extent the activity of salts is related to their ability to interact with proteins. RNA may be extracted from tissues using salts with predominantly hydrophilic properties and DNA with salts having lipophilic properties.

While protein partitions principally in the phenol phase, a second extraction is required to yield nucleic acids of high stability and purity (Kirby 1965).

Phenol alone is not completely successful in removing protein and a mixture of phenol and *m*-cresol is a better deproteinizing agent. Phenol with 8-hydroxyquinoline was used by Kirby (1962) to prevent oxidation and discoloration. He found that some salts, previously ineffective, released DNA with phenol–8-hydroxyquinoline. The difference is probably related to the removal of metal linkages from the nucleoprotein structure by the 8-hydroxyquinoline.

The nature of the intermolecular bonding and association varies in different tissues so that no general method for the isolation and purification of nucleic acids can necessarily be expected. However, the successful application of a particular method is itself an indication of the molecular architecture present in the cells investigated.

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The present study was undertaken to investigate the nucleic acids of wool roots and to find the optimum methods for their extraction from this highly specialized and differentiated tissue.

Little is known of the mechanism of protein biosynthesis in the wool root and a basic study of the protein-synthesizing machinery in this tissue is fundamental to such an investigation.

The ribosomes and polysomes of the wool root have been characterized (Wilkinson 1970). A similar study on the nucleic acids has now provided a firm base for investigating the role of these cell components in keratin biosynthesis.

II. Materials and Methods

Wool roots were procured from sheep by the method previously described (Wilkinson *et al.* 1969; Wilkinson 1970) and nucleic acids were extracted from whole and fractionated wool roots by the following procedures.

(*a*) *Extraction of Nucleic Acids from Whole Wool Roots*

(i) *Ribosomal RNA* (Kirby 1965, Method 1)

(1) Wool roots stripped from sheep were immediately harvested from the fibre-glass tape by Oster clippers and dropped into ice-cold 0·5% disodium naphthalene-1,5-disulphonate (15 vols. w/v; Eastman Organic Chemicals, N.Y.) and shaken for 5 min at 4°C on a wrist-action shaker. An equal volume of phenol–cresol mixture (500 g phenol crystals, 70 ml *m*-cresol, 0·5 g 8-hydroxyquinoline, 55 ml water) was added and the mixture shaken for 15 min at room temperature. The two phases were separated by centrifuging at 12,000 *g* for 20 min at 4°C.

(2) The aqueous phase was removed by pipette and made 5% with respect to sodium triisopropylnaphthalenesulphonate (Eastman Organic Chemicals, N.Y.). This was then shaken with 0·5 vol. of phenol–cresol mixture for 15 min at room temperature and centrifuged at 12,000 *g* for 10 min at 4°C.

(3) The top phase was removed by pipette and made 3% with respect to sodium chloride and 20% with respect to sodium benzoate. After dissolution, 0·1 vol. of *m*-cresol was added. The precipitate of ribosomal RNA (*r*RNA), which formed immediately on shaking, was separated by centrifuging, washed twice with an ice-cold mixture of 100 ml water, 3 g sodium chloride, 20 g sodium benzoate, and 10 ml *m*-cresol, once with a cold mixture of 30 ml water, 1 g sodium chloride, and 70 ml ethanol, and finally twice with 70% ethanol. The *r*RNA was either dissolved in 0·1M sodium acetate buffer, pH 6·0, or washed once with ethanol and dried *in vacuo* over calcium chloride.

(ii) *Soluble RNA*

The top phase (3) in the second extraction step of the above technique contained *r*RNA and soluble RNA (*s*RNA) (and polysaccharide). *s*RNA was isolated from the mixture by precipitation of the whole with 2 vols. of ethanol. After the precipitate had been washed twice with 70% ethanol and dissolved in 0·1M sodium acetate buffer, pH 6·0, the *s*RNA was freed of *r*RNA by making the acetate buffer 3M with respect to sodium chloride. The *r*RNA precipitated out overnight at 4°C and was centrifuged off. Sodium chloride was dialysed out of the *s*RNA solution against 0·1M sodium acetate buffer, pH 6·0.

Alternatively, *s*RNA could be extracted from the residue left after precipitation of *r*RNA with *m*-cresol (above), providing the triisopropylnaphthalenesulphonate was first removed as the potassium salt.

(iii) *DNA* (Kirby and Cook 1967)

(1) Wool roots were shaken in 25 vols. (w/v) of an aqueous solution containing 1% sodium triisopropylnaphthalenesulphonate, 6% s-butanol, 6% sodium 4-aminosalicylate (B.D.H.), and 1% sodium chloride for 5 min at 4°C, and an equal volume of phenol–cresol mixture was

added. Shaking was continued at room temperature for 20 min and the phases were then separated by centrifuging at 12,000 *g* for 20 min at 4°C.

(2) The top phase was made 3% with respect to sodium chloride and shaken with 0·5 vol. of phenol–cresol mixture for 15 min at room temperature.

(3) The top phase, separated by centrifuging, was made 20% with respect to sodium benzoate, and 2-butoxyethanol was added to 0·1 vol. The DNA and some RNA precipitated as a gel. This was centrifuged off, dissolved in 0·1M sodium acetate buffer, pH 6·0, and reprecipitated by the addition of sodium chloride to 3%, sodium benzoate to 20% and 2-butoxyethanol to 0·2 vol.

(4) This precipitate was washed with 75% ethanol and dissolved in 0·1M sodium acetate buffer, pH 6·0. RNA was removed by the addition of sodium chloride to 3M and allowing it to precipitate overnight at 4°C. The DNA was precipitated from the supernatant solution by the addition of 1 vol. of 2-ethoxyethanol. The fibrous precipitate was washed twice with 75% ethanol and twice with ethanol and was then either dissolved in 0·1M sodium acetate buffer, pH 6·0, or dried *in vacuo* over calcium chloride.

(iv) *Rapidly Labelled RNA* (Parish and Kirby 1966)

Two volumes of cold ethanol were added to the top phase (3) of the second extraction step of the preparation of DNA; the nucleic acids precipitated and were collected by centrifuging after standing for 2 hr at 4°C. The precipitate was washed twice with 70% ethanol and dissolved in 0·1M sodium acetate buffer, pH 6·0. The solution was made 3M with respect to sodium chloride and left overnight at 4°C. The RNA which precipitated was centrifuged out, washed once with 3M sodium acetate buffer, pH 6·0, and twice with 70% ethanol, and dissolved in 0·1M sodium acetate buffer, pH 6·0.

(*b*) *Extraction of RNA from Wool Root Fractions*

(i) *Ribosomes and Polysomes*

Ribosomes and polysomes, pelleted by centrifugation of cell supernatant fractions over 1M sucrose in standard buffer (Wilkinson 1970), were dissolved in 1% sodium dodecylsulphate in 0·1M sodium acetate buffer, pH 6·0, by incubation at 37°C for 2 min (Burny and Marbiax 1965) and analysed by sucrose density-gradient centrifugation.

(ii) *Cell Debris*

The residue remaining after withdrawing the cell supernatant fraction from the 12,000 *g* centrifugation of homogenized wool roots in standard buffer (Wilkinson 1970) was subjected to the same extraction procedure as for Section II(*a*)(iv) above.

(*c*) *Base Analyses*

(i) *RNA*

RNA (1·5 mg) was hydrolysed to purine bases and pyrimidine nucleotides by heating in a sealed tube at 100°C with 0·05 ml 1N HCl for 60 min. 0·01-ml aliquots of the hydrolysate were spotted in triplicate on Whatman No. 1 chromatographic paper and the bases separated by upward chromatography using the solvent system methanol 50%, ethanol 25%, water 19%, conc. HCl 6% (Kirby 1965). The chromatogram was run overnight and the papers dried at room temperature until free from acid. The spots, defined by ultraviolet light, were cut out and together with corresponding blanks were eluted over night with the following solvents: guanine, 5 ml 0·5N HCl; adenine, 5 ml 0·1N HCl; cytidylic acid, 5 ml 0·2N sodium acetate; uridylic acid, 5 ml 0·2N sodium acetate.

The ratios of the molar concentrations of the purines and the pyrimidine nucleotides were determined by measuring the respective absorptions in 1-cm silica cells (Beckman D. B. G. spectrophotometer) at λ_{max}: guanine 249 nm; adenine 260; cytosine 269; uridine 261.

The base ratios were calculated as moles base per 100 moles total bases.

(ii) *DNA*

DNA (2·5 mg) was hydrolysed with 0·5 ml 90% formic acid in a sealed tube heated at 176°C (bath of boiling dichlorobenzene) for 1 hr. The tube was opened after being frozen in liquid

nitrogen, the contents transferred to a small flask, and the formic acid removed by rotary evaporation. The residue was dissolved in 0·1 ml 1N HCl and 0·01 ml was taken for paper chromatography (Whatman No. 4) using the solvent system methanol 70%, conc. HCl 20%, water 10%. After development for 16 hr the spots were marked, cut out, and eluted with 5 ml 0·1N HCl for adenine, cytosine, and thymine, and 5 ml 0·5N HCl for guanine (Kirby 1957).

(*d*) *Phosphorus Determination*

Solutions of RNA and DNA of known optical density were digested with 2 ml perchloric acid (60%) under reflux for 2 hr. The colourless solutions were quantitatively transferred to volumetric flasks and 2 ml amidol reagent (2 g amidol and 40 g sodium bisulphite in 200 ml water) were added, followed by 1 ml ammonium molybdate reagent (8·3% in water) and water to 25 ml (Allen 1940). The optical densities at 760 nm of the test solution, a blank (water), and a standard (1·0967 g potassium dihydrogen phosphate in 250 ml water) were measured between 5 and 30 min after making up to volume. Phosphorus concentrations were calculated by proportions.

(*e*) *Protein Contamination of RNA and DNA*

Nucleic acids (10 mg) were hydrolysed in sealed tubes with 0·5 ml 5·8N HCl at 100°C for 16 hr (RNA) or 24 hr (DNA). The tubes were opened, the solutions evaporated, and the residues washed three times with 0·5 ml water. The residues were finally taken up in sodium citrate buffer, pH 2·2, and the amino acids analysed on a Beckman 120C amino acid analyser.

(*f*) *RNA Contamination of DNA*

DNA (5 mg) was hydrolysed with 0·05 ml 1N HCl in a sealed tube for 1 hr at 100°C. A portion of the solution (0·01 ml) was chromatographed on Whatman No. 4 paper with the solvent system methylethylketone 60%, glacial acetic acid 20%, water 20% (Kirby 1957). A standard solution of ribose (0·3%) was run concurrently and ribose was detected by spraying the paper with the aniline hydrogen phthalate reagent of Partridge (1949) (aniline 0·93 g, phthalic acid 1·66 g, water-saturated n-butanol 100 ml) and heating at 100°C for 5 min.

(*g*) *Glycogen Contamination of RNA and DNA*

Nucleic acids (5 mg) were treated as in Section II(*f*) for the detection of glucose, a standard solution of glucose (0·3%) being run as a reference.

(*h*) *DNA Contamination of RNA*

RNA (2·7 mg) was dissolved in water (1 ml) and an equal volume of 10% trichloroacetic acid added; the mixture was boiled for 30 min in a graduated tube. The solution was cooled rapidly, made up to volume, and divided into two. To each part was added 0·05 ml of freshly prepared *p*-nitrophenylhydrazine reagent (0·5% in 95% ethanol) (Webb and Levy 1955). A blank with trichloroacetic acid alone was made up and control samples with known weights of purified DNA were also run. The tubes were placed in a boiling water-bath for 20 min, cooled, 2·5 ml n-butyl acetate added, and shaken vigorously. The phases separated on standing or after light centrifugation and 0·3 ml of the bottom phase was removed by pipette. 0·1 ml of 2N NaOH was added and the colour measured at 560 nm after 1 min.

(*i*) *Other Methods*

(i) *Sucrose Density-gradient Centrifugation*

Ribonucleic acids were routinely analysed by sucrose density-gradient centrifugation. Gradients were linear from 5–20% sucrose in 0·01M sodium acetate buffer, pH 6·0, and were centrifuged at 22,000 r.p.m. for 16 hr at 4°C in the SW25·1 rotor of a Spinco L4 ultracentrifuge. They were emptied by upward displacement and continuously monitored at 254 nm with an ISCO model 222 ultraviolet analyser. Fractions were measured manually at 260 and 280 nm or

were scanned from 220 to 320 nm using a Beckman DBG spectrophotometer. Radioactive samples were counted in a Beckman LS200 liquid scintillation spectrometer either by adding directly to Bray's solution or by co-precipitating with RNA, washing on to Millipore filters (HAWP 02500; HA 0·45 μm) and counting in toluene scintillating fluid (toluene 1 litre, dimethyl POPOP 0·2 g, PPO 4 g).

(ii) *Radioactive Labelling of RNA*

Radioisotopes were obtained from the Radiochemical Centre, Amersham, England. Tritiated uridine (G) (2·70 Ci/m-mole) was injected into hyaluronidase-treated skin (Wilkinson 1970) and sodium [^{32}P]orthophosphate (5 mCi/ml) was injected intravenously.

(iii) *Melting Temperature of Ribosomal RNA and DNA*

Thermal denaturation was achieved by using the heated cell block of a Beckman DBG spectrophotometer and solvent denaturation was accomplished with 2-methoxyethanol at 0–80% at constant salt concentration.

III. Results

(a) *Ribosomal RNA from Whole Wool Roots*

Ribosomal RNA was extracted from whole wool roots in good yield (7·5 mg/g wool roots) with no contamination from DNA, *s*RNA, or polysaccharide. The sucrose gradient profile is shown in Figure 1. The resolution between the 28S and the 18S peaks was good and the ratio 28S : 18S was 1 : 2.

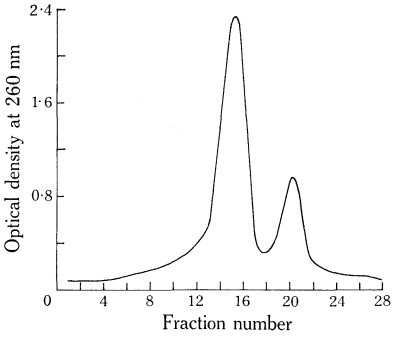

Fig. 1.—Sucrose density-gradient profile of ribosomal RNA from wool roots. Fractions were dripped out from the bottom of the tube and the optical densities measured manually. Sedimentation is to the left.

Protein contamination was 0·084%, calculated (D. D. Haden, personal communication) from the chart recording of the Beckman 120C amino acid analyser.

Analysis of the RNA gave a phosphorus content of 3·48 μg per optical density unit and an $E^{1\%}_{1cm}$ of 244. Base composition (corrected for 5% loss on hydrolysis of cytosine and uracil) was guanine : adenine : cytosine : uracil = 33·7 : 17·8 : 33·1 : 15·4.

The percentage phosphorus, based on the data above, was 8·49% (w/w), while the theoretical phosphorus, considering base composition and correcting for sodium salt of the RNA, was 8·53%.

The atomic extinction coefficient with respect to phosphorus, E(P), at 260 nm (Chargaff and Zamenhof 1948) was 8903 in water.

The ultraviolet spectrum of the RNA showed λ_{max} to be 258 nm and λ_{min} to be 232 nm; the ratio E_{260nm}/E_{280nm} was 2·20.

The melting temperature (T_m) of RNA in 0·15M sodium chloride–0·015M sodium citrate was 44·0°C and the mid-point of the melting curve of hyperchromicity against concentration of 2-methoxyethanol (at 0·1M sodium chloride) was obtained at 36% 2-methoxyethanol with maximum net increase in E_{260nm} of 22·4% obtained at a solvent concentration of 75%.

(*b*) *Ribosomal RNA from Wool Root Fractions*

Sucrose density-gradient profiles showed the same high resolution between the 28S and 18S peaks (Fig. 3) as was obtained for *r*RNA from whole wool roots (Fig. 1). The ratio 28S to 18S was again 1 : 2 for each fraction.

Sucrose-gradient fractions, measured manually (27 fractions of 1 ml), resolved the 28S and 18S peaks only (Fig. 3). Continuous optical density monitoring with a flow-through cell resolved minor peaks shown in Figure 2, as follows:

Total r*RNA from Whole Wool Roots.*—A small peak of 4–5S RNA and a shoulder on the leading edge of the 28S peak were evident.

r*RNA from Cell Debris.*—A trace of 4–5S RNA was apparent and the shoulder on the 28S peak was resolved into a small peak, possibly because of the better resolution achieved with less loading of the sucrose gradient.

r*RNA from the Cell Supernatant Fraction.*—The preparation from ribosomes and polysomes isolated from the cell supernatant fraction showed no shoulder or peak on the leading edge of the 28S RNA peak and the presence (or absence) of 4–5S RNA was masked by the ultraviolet absorption of the sodium dodecylsulphate at the top of the gradient.

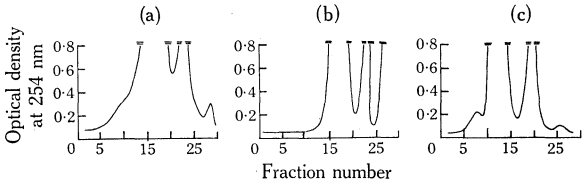

Fig. 2.—Sucrose density-gradient profiles of RNA (plus rapidly labelled RNA) (*a*) from whole wool roots (total RNA), (*b*) from the supernatant fraction obtained by centrifuging wool root homogenates in standard buffer (Wilkinson 1970) at 12,000 *g* (cell supernatant fraction RNA), and (*c*) from the residue after withdrawing the supernatant fraction (cell debris RNA). Sucrose gradients were emptied by upward displacement and optical densities were continuously monitored. The optical densities were not recorded above 0·8, but the incomplete profiles are shown to illustrate the presence of a minor component on the leading edge of the 28S *r*RNA peak (see text). Sedimentation is to the left.

The sucrose-gradient profiles of RNA from whole wool roots, cell debris, and the cell supernatant fraction were cut out, weighed, and the weights related to the initial weights of material. The RNA from cell debris (nuclear?) was slightly less than half that from the cell supernatant fraction (cytoplasmic?) ($E_{260nm} = 0·115$ compared with $E_{260nm} = 0·30$), and taken together the amount of *r*RNA from these two fractions equalled that extracted from whole wool roots. Approximately 32% of *r*RNA in wool roots was extracted from the cell debris and 68% from the cell supernatant fraction.

(c) *Radioisotope Labelling*

As for Section III(*b*), RNA was extracted from whole wool roots, cell debris, and the cell supernatant fraction after labelling with sodium [^{32}P]orthophosphate for 30 min, 4, and 24 hr (cf. Wilkinson 1970). Preliminary studies were undertaken with [^{3}H]uridine labelling of RNA from whole wool roots at 15, 30, and 60 min.

(i) ^{32}P *Labelling*

No significant activity was obtained in the sucrose gradients of RNA from the three sources except at 24 hr (Fig. 3). The specific activity was particularly low and there was no evidence of labelling ribosomal RNA precursor or messenger RNA.

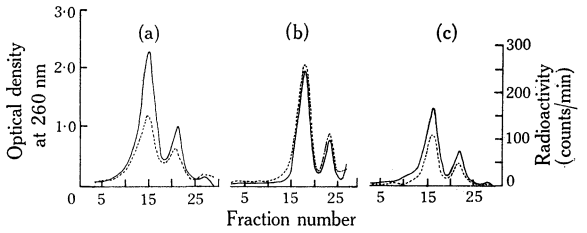

Fig. 3.—Sucrose density-gradient profiles of ribosomal RNA (plus rapidly labelled RNA) (*a*) from whole wool roots (total), (*b*) from the cell supernatant fraction, and (*c*) from cell debris (cf. Fig. 2). Gradients were emptied by upward displacement but optical densities (——) were measured manually. The radioactive profiles (– – –) show the incorporation of sodium [^{32}P]orthophosphate 24 hr after injection. At times less than this, the activity was not significant (for details see text).

(ii) [^{3}H]*Uridine*

The results from the [^{3}H]uridine incorporation study appear to conflict with those from ^{32}P labelling (Fig. 4), but it may well be that the specific activity of the RNA fractions in the ^{32}P study was too low to resolve minor trends in the labelling profile. The [^{3}H]uridine-labelled RNA gradients were dripped out manually and the "pellets" at the bottom of the tube were assayed. The ^{32}P gradients were emptied by upward displacement through a continuous flow system, however, and the "pellets" were not recovered.

It is possible that the radioactive profiles in Figure 4 represent a more accurate description of RNA synthesis in wool roots at short pulse times, though the significance of the high activity at the bottom of the gradient is not clear. While giant-size rapidly labelled nuclear RNA has been reported in other tissues (Attardi *et al.* 1966), there appeared to be no simple precursor-to-product-type relationship between the heavy nuclear RNA and cytoplasmic messenger RNA. Limited evidence in this report and previous work (Wilkinson 1970) tends to suggest that the heavy RNA, labelled at short pulse times, is not messenger RNA.

The remaining radioactivity in the [^{3}H]uridine gradients at 15 and 30 min pulse times is probably ribosomal RNA precursor and at 60 min the majority of the label is incorporated into ribosomal RNA.

(d) *Soluble RNA*

Soluble RNA was extracted from whole wool roots with a yield of 1 mg/g wool roots. Only a trace of ribosomal RNA was present as shown by sucrose-gradient

centrifugation. No DNA was present, but the preparation was contaminated by polysaccharide. The sedimentation coefficient was 4–5S (assuming 18S and 28S for ribosomal RNA).

(*e*) *DNA*

DNA was extracted from whole roots with a yield of 1 mg/g wool roots. Protein contamination was 0·06% (D. D. Haden, personal communication), and the hydrolysate was not characterized by high lysine or arginine. No quantitative measurements of RNA and polysaccharide were made, but chromatography of HCl hydrolysates of DNA showed slight contamination from RNA and very slight contamination from polysaccharide. The ribose and glucose spots were very much fainter than the controls of ribose and glucose at 0·3%.

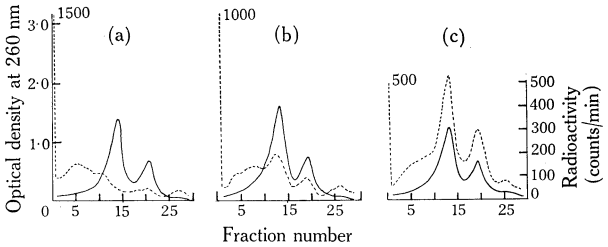

Fig. 4.—Sucrose density-gradient profiles of ribosomal RNA plus rapidly labelled RNA from whole wool roots. Gradients were emptied from the bottom of the tubes and the optical densities (——) measured manually. The radioactivity profiles (– – –) show the incorporation of [^{3}H]uridine with pulse times (min) of 15 (*a*), 30 (*b*), and 60 (*c*). The activity in the "pellets" is shown at the top left of each profile.

The phosphorus content was 4·08 μg per optical density unit, the $E^{1cm}_{1\%}$ was 232, and E(P) in water was 8150. Base composition (moles/100 moles total bases) was guanine : adenine : cytosine : thymine = 22·5 : 28·8 : 24·8 : 23·9.

Thermal denaturation of DNA in 0·15M sodium chloride–0·015M sodium citrate gave a melting temperature of 85°C. Solvent denaturation (at 0·1M sodium chloride) using 2-methoxyethanol gave a melting mid-point at 45% and a maximum increase in E_{260nm} of 28% obtained at 75% organic solvent.

IV. Discussion and Conclusions

Extraction of the major nucleic acid species from wool roots has been accomplished using techniques previously applied to endocrine organs and transplantable tumours of the rat (Kirby 1965; Parish and Kirby 1966; Wilkinson and Kirby 1966*a*; Kirby and Cook 1967) and to rabbit reticulocytes and fractionated cells from rabbit bone marrow (Hunt and Wilkinson 1967). Few modifications appeared necessary to obtain nucleic acids in good yield and purity. The proportion of extracting medium to the weight of tissue was increased to allow for the greater "bulkiness" of wool roots, as much of the material was keratinized and resistent to the extracting media. It was essential to extract the wool roots in the aqueous phase for a short time before the addition of the phenol–cresol–8-hydroxyquinoline mixture, otherwise

yields were markedly diminished, presumably because of denaturation of protein prior to breaking the cells. The use of tissue blenders and rapid stirring and freezing was avoided to limit shear.

Earlier studies (Kirby 1957, 1958, 1959), with phenol and different salts in the aqueous phase have shown that hydrophilic salts release little or no DNA, but liberate *r*RNA. Lipophilic salts release DNA to which some protein remains bound, and salts that have both lipophilic and chelating properties release DNA (and RNA) with minimal protein contamination. Naphthalenedisulphonate is a suitable salt for extracting RNA, as it has highly charged hydrophilic groups at opposite ends of the molecule, while 4-aminosalicylate is a very satisfactory salt for extracting DNA, having both lipophilic and chelating properties. Triisopropylnaphthalenesulphonate is a more suitable detergent than sodium dodecylsulphate as it can be used with phenol at higher concentrations without forming a single-phase system.

The phenol–cresol–8-hydroxyquinoline mixture (Kirby 1965) is a more satisfactory deproteinizing agent than phenol alone and ribonuclease is known to survive phenol treatment (Huppert and Pelmont 1962; Littauer and Sela 1962).

The analyses of ribosomal RNA and DNA indicate that they are not grossly different from those from other mammalian tissue. Degradation has been avoided and contamination of the preparations by protein, polysaccharide, and other nucleic acid species was minimal. Though glycogen contamination was apparent in DNA preparations, this could be markedly reduced by the centrifugation or differential extraction methods of Kirby (1964).

The test for DNA (Webb and Levy 1955) is particularly sensitive to low concentrations of DNA and may be performed very rapidly. It would thus be a useful method for estimating the amount of wool roots in a sample rather than by weight as it is difficult to achieve reproducible clipping of wool roots from the fibre-glass tape (Wilkinson 1969).

The isotope labelling data support the conclusions from a previous study (Wilkinson 1970) that messenger RNA in wool roots is probably stable. There was no evidence of a rapidly labelled heterogeneous RNA fraction in preparations from wool roots extracted by methods shown to isolate ribosomal RNA together with rapidly labelled RNA from rat liver (Kirby 1965; Parish and Kirby 1966) and from bone marrow cells (Hunt and Wilkinson 1967) and haemoglobin messenger RNA from rabbit reticulocytes (Burny and Marbiax 1965). At least a part of the rapidly labelled RNA fraction had messenger properties (Wilkinson and Kirby 1966*b*; Parish and Kirby 1966). The data in the present study are not definitive, particularly as there is some discrepancy between the [^{3}H]uridine and sodium [^{32}P]phosphate studies, but the labelling profile in Figure 4 is strongly suggestive of ribosomal RNA precursor labelling (Perry 1962; Scherrer, Lathan, and Dornell 1963) at the shorter pulse times.

Preparations of *r*RNA plus rapidly labelled RNA from whole wool roots and cell debris contained traces of a component which sedimented on the leading edge of the 28S *r*RNA (Fig. 2). This component was not present in the preparation from ribosomes and polysomes. The method of extraction in the latter case was different but it may be significant that the component was present only in preparations containing nuclear material. While the colorimetric tests of DNA contamination of

RNA were negative, the assay may not have been sufficiently sensitive to detect this minor component if it was in fact DNA. It was probably not messenger RNA as it did not appear in gradients of RNA extracted from ribosomes and polysomes. There is approximate coincidence between the position of the component in sucrose gradients and that of radioactive RNA labelled at short pulse times (Fig. 4), which suggests that the component may be ribosomal RNA precursor.

Whatever the nature of the component, it is apparent that continuous monitoring of optical density profiles in sucrose gradients of RNA (and of other materials) is a more sensitive method for detecting minor components than the manual "drip-out method" usually employed.

The present study has shown that the major species of nucleic acids may be isolated from wool roots and wool root fractions in good yield and purity and that the preparations are comparable with those from other mammalian tissues.

The role of nucleic acids and polysomes in the biosynthesis of wool keratin will be reported in further papers in this series.

V. Acknowledgments

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